6333

FINAL REPORT ON

CONTRACT NO DA CRD-AG-892-544-62-G2

INCLUSIVE DATES 23 February 1962 TO 22 February 1963

SUBJECT OF INVESTIGATION

RELATION BETWEEN
THE ELECTRORETINOGRAM (ERG)
AND
SINGLE CELL ACTIVITIES
IN THE RETINA

RESPONSIBLE INVESTIGATOR

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U.S. Army Research & Development Group (9852) (Far East)
Office of the Chief of Research and Development
United States Army
APO 343

AD Div 16/1

School of Medicine, Keio University, Tokyo (Japan) RELATION BETWEEN THE ELECTRORETINOGRAM (ERG) AND SINGLE CELL ACTIVITIES IN THE RETINA by Tsuneo Tomita. Final Report, 23 Feb 62 - 22 Feb 63. 16 p. illus. tables, 19 refs. (Grant DA-CRD-AG-S92-544-62-G2) Unclassified Report.

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UNCLASSIFIED

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2. Retina Frogs - Fishes

I. Title: None
II. Tomita, Tsuneo
III. U. S. Army R&D Gp (FE), OCRD, DA, Wash, D. C.
IV. Grant No. DA-CRD-AG-S92-544-62-G2

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RELATION BETWEEN THE ELECTRORETINOGRAM (ERG) AND SINGLE CELL ACTIVITIES IN THE RETINA

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ABSTRACT

Attempt at identifying the cell types that make sources of the ERG components is continued from our work described in Final Report on U.S.Army Research Grant No. MG1-60-1. Of the three groups of responses (Group-I, -II and -III) recorded from single units in the frog's retina, only the Group-II responses were left suspected in the preceding report to be constituents of the ERG, but the present work excludes this possibility, since it has become most probable that the responses in question arise from the soma and/or dendrites of ganglion cells which are known to contribute little to the ERG. The failure to record any constituents of the ERG in the frog has led our research in two directions. The one is the effort to make intracellular recording possible, by some further technical improvement, from cell types other than those already studied but proved to contribute little to the ERG. A technique of "harpooning" single cells with pipets at high momentary acceleration is being tested and will make a subject in the coming year. The other is to apply the conventional technique to materials other than the frog and see whether the localization of ERG constituents is possible in these materials. Among several animals so far examined, the carp is subject to a more thorough test in this report. Studied are properties of Group-III responses (S-potentials), and the spatial distribution of ERG components in the focal and non-focal regions of the retina.

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CONTENT PAGE

I. Introduction 1

II. Method 3

1. Photostimulator

2. Micropipet Electrode, Amplifier and Recording System

3. Material

4. Chemicals

III. Results 5

1. Experiments on the Frog's Retina

a. The Cell Type Yielding Group-II Responses

b. Localization of PIII by Means of Sodium Azide

2. Extension of Experiments to Other Cold-blooded Retinas

a. Comparison of the Degree of Ease of Intracellular Recording from Different Materials

b. Group-III Responses (S-potentials) in the Carp

3. Component Analysis of the Fish ERG

a. ERG's in Response to Focal and Non-focal Illumination, and Their Depth Recording

b. Distribution of ERG Components in the Focal and Non-focal Retinal Regions --- Presentation by a Model

c. Evidence for the Model

(1) Effect of Small Indifferent Electrode

(2) Effect of Some Chemicals

IV. Discussion 12

V. Summary 13

VI. References 14

VII. Illustrations 16

I. INTRODUCTION

The work to be described in this report is the continuation from that furnished in the Final Report on Research Grant No. MG1-60-1 (Responsible Investigator, Tsuneo Tomita), inclusive dates 22 April 1960 to 21 October 1961. Before going into the detail of the present work, it may be helpful to summarize our earlier work as the background.

By the use of very minute micropipets, the diameter of which was less than 0.1μ, it became possible to record intracellularly the response of single cells in the frog's retina. The response types most frequently obtained were classified into three; Group-I, -II and -III. The Group-I responses which were obtained from the most superficial retinal layer comprised impulses of either on-, off-, or on/off-type, but were accompanied by no discerned slow potential change. The Group-II responses which were found from a layer lying some 70 to 80μ deeper than the depth at which the Group-I responses were recorded also comprised impulse discharges similar to the Group-I responses, but they were featured by impulses superimposed on conspicuous slow potential changes resembling the ERG or some components of it, that is the a-, b- or/and d-wave. No impulse activity was detectable beyond the depth at which the Group-II responses were found, but there existed a layer from which a sustained negative potential was obtained in response to illumination (Group-III). The origin of the Group-I responses was identified to be single optic nerve fibers. The Group-III responses were also identified as the S-potential which was first observed by Svaetichin (1953) in the fish retina and had since been studied extensively. With regard to the Group-II responses which were suspected to be constituents of some ERG components, several attempts to identify the responsible cell type were made by means of antidromic stimulation of the optic nerve, by application of polarizing current across the retina, and by use of some chemicals. Either one of the two cell types, the ganglion cell or bipolar cell, appeared to be responsible for the Group-II, but a final conclusion was left open to further investigations.

In the present report, evidence will be provided that the cell type that produces Group-II responses is not the bipolar cell but the ganglion cell. This will then be followed by description of our attempt at intracellular recording from materials other than the frog, particularly from the fish. As is well known, the Group-III responses, or

S-potentials in the fish are large and easily obtainable. They consequently disturb the depth recording of the ERG, making the component analysis far more difficult than in the frog. Since, on the other hand, some of the fish S-potentials are related to color reception mechanisms (MacNichol and Svaetichin, 1958), it was thought that this might make an advantage for the understanding of the retinal mechanism.

II. METHOD

1. PHOTOSTIMULATOR

In addition to the one described in the preceding report with Bausch & Lomb's grating monochromator as its principal part, the photostimulator shown schematically in Fig.1 and described by Tomita et al. (1960) was also used. For alternate focal and non-focal illumination with this photostimulator, for instance, the diaphragm in one channel is a metal plate provided with a hole, the light image of which is focussed on the retinal region where the recording electrode is situated (focal illumination), while the diaphragm in the other channel is a glass plate with a round piece of black paper pasted on it to make a dark image on the recording retinal site (non-focal illumination).

2. MICROPIPET ELECTRODE, AMPLIFIER AND RECORDING SYSTEM

All of these were the same as in the preceding report in but one point: The crosscompensation device necessary for our coaxial type microelectrode has been much improved as the result of replacement of several vacuum tubes in the old device by transistors (Tomita, 1962).

3. MATERIAL

The material was extended from the frog, the principal material in the preceding report, to the carp, Cyprinus carpio, and also to the turtle, Geoclemys reevesii. The preparation was either the excised opened eye or the retina detached from the pigment epithelium, depending on the purpose of experiment.

4. CHEMICALS

Sodium azide is known as a depressant of PII component in the ERG (Noell, 1953; Müller-Limmroth and Blümer, 1957), and ethyl alcohol as a PIII depressant (Bernhard and Skoglund, 1941). 0.01 to 0.1% sodium azide in Ringer, and 10% alcohol in Ringer were used for the component analysis of the ERG. In some experiments, however, alcohol vapor in air coming out of a fine injection needle was applied onto the retina. The duration of vapor application was controlled by switching on and off of a small vibration type air pump which supplied air bubbles through liquid alcohol. The air-

alcohol vapor mixture thus obtained was collected to come out of the needle. The application of another PII depressant, ammonia, was exclusively in gaseous form, using the same device as in alcohol. The application of chemicals in gaseous form had one big advantage: The recording micropipet within the retina could be kept located exactly at the same site before and after the chemical application.

III. RESULTS

1. EXPERIMENTS ON THE FROG'S RETINA

a. The Cell Type Yielding Group-II Responses

It was mentioned in the preceding report that among the three groups of responses recorded from within the frog retina by very minute micropipet electrodes two of them, Group-I and -III, were disqualified for candidates of ERG constituents. Only the Group-II responses were left open to further tests with the suspicion that they might take part in forming the ERG. My conclusion now is that the contribution of the Group-II responses to the ERG is little, if any, since these responses most probably originate from ganglion cells that are known to do little to the ERG. This is supported by our earlier observation that the Group-II neurons are invaded by antidromic impulses elicited by stimulation of the optic nerve. One shortcoming might be that the depth at which the Group-II responses are obtained does not correspond to the ganglion cell layer, but it is somewhere between the inner plexiform layer and inner nuclear layer. However, there is evidence that the ganglion cells, like other cells, are impaled at their soma or dendrites only after they have been pressed down a certain distance by micropipet electrodes. This may account for failure to record from the depth corresponding to the ganglion cell layer. This may also be in accord with the observation that all the three groups of responses were localized close to the inner limiting membrane when pipets were inserted from the opposite side of the retina, that is from the receptor side. There are also several other pieces of evidence. First, the slow potential changes in Group-II responses are what one can predict in the ganglion cells from their three types of responses which have been known since Hartline (1938). The result of intracellular recording by Wiesel (1959) from ganglion cells in the cat also shows that the pattern of impulse discharge depends entirely on the slow potential changes recorded simultaneously. Second, Brown and Wiesel (1959) report that the units responding with impulse discharges, found in the cat from a depth corresponding to the inner nuclear layer, change their response type in the same manner as observed in the ganglion cells by Kuffler (1953). Third, although this observation of Brown and Wiesel was not confirmed in our experiment on the frog, since the response type of each ganglion cell in the frog is far more fixed

than in the cat, comparison of the receptive fields of Group-I responses which are from ganglion cell axons and Group-II responses in question disclosed no substantial differences. It is probable, therefore, that both responses are obtained from different regions of the same cell type, the ganglion cell.

b. Localization of PIII by Means of Sodium Azide

Discussions were made in the preceding report on the contradiction concerning the origin of PIII, or the a-wave in the ERG, between the frog studied by us and mammals studied by Brown and Wiesel (1961) and Brown and Watanabe (1962). While the major source of the frog's PIII is localized by us in the bipolar cell layer, Brown and his coworkers hold the view that the PIII in mammals originates exclusively in the receptor layer. Under such circumstances, there occurred the possibility that the PIII might be made up of more than one component from different retinal layers and that in different animal forms the component that dominates might differ. The following experiment appears to materialize this possibility.

The arrangement is shown in Fig.2. A coaxial microelectrode is applied to the frog's opened eye with its outer pipet in contact with the inner retinal surface to record the surface ERG through one channel of a two-channel amplifier. The inner superfine pipet which is connected to the other channel of the amplifier protrudes out of the tip of the outer pipet into the retina to a minimal depth at which the intraretinally obtained ERG is just reversed in polarity (lower tracing in Fig.3a). It is indicated by this depth that the inner pipet has just penetrated through layers producing the major portion of ERG to the opposite side (Tomita, Murakami and Hashimoto, 1960). The depth of the inner pipet in the example illustrated in Fig.3 was 140μ from the vitreal retinal surface. The inner pipet, as well as the outer pipet, was fixed at such positioning throughout the experiment. Fig.3a is a control record from a preparation with the vitreous humor drained off, the upper tracing being the surface ERG. Record (b) was obtained after filling the eye cup with Ringer solution, showing no substantial change from (a). Subsequent records were obtained 2 minutes (c), 4 minutes (d), and 6 minutes (e) after replacement of Ringer solution in the eye cup with 0.1% azide-Ringer, a PII depressant. Component PIII soon becomes dominant in both surface and intraretinal ERG's (c), but since both tracings are opposite in polarity at this early stage of azide action, the e.m.f. for the ERG in which PIII is now dominant is

undoubtedly in some layers that intervene between the two pipets. In the course of time, however, both surface and intraretinal ERG's become small again (d), and eventually the intraretinal ERG takes the same polarity and amplitude as the surface ERG (e), indicating clearly that the e.m.f. responsible for this remaining response no longer exists in layers between the two pipets but somewhere else. Since, by further advancement of the inner pipet across the receptor layer, the response from the inner pipet reverses its polarity again to show a mirror image of that from the outer pipet (not illustrated), it is concluded that this portion of PIII which survives azide originates more distally than all the rest of the ERG in which a large portion of PIII is involved. It is probable that this fraction of PIII having a more distal origin arises from receptors, although it may remain to be solved why the polarity of PIII is just the opposite of what one expects from other types of receptors, as earlier discussed by Granit (1947). While it is general that the distal tip of the receptor becomes negative when excited, the component PIII is in the direction that makes the distal tip positive instead of negative.

2. EXTENSION OF EXPERIMENTS TO OTHER COLD-BLOODED RETINAS

a. Comparison of the Degree of Ease of Intracellular Recording from Different Materials

It was discouraging that all the responses recorded in good isolation from minute pipets in the frog's retina were disqualified as candidates of ERG constituents. One may have to look for some other means for successful intracellular recording from retinal cells other than those already studied by our minute pipets. Or, it might as well be worthy of trying on materials other than the frog. For the former, we have just started testing the possibility of "harpooning" single cells with a pipet in momentary motion at high acceleration. For the latter, we have tried the carp, eel, turtle and newt. Only the carp and turtle were found adequate for this kind of experiment. Responses were obtained intracellularly from the eel and newt as well as from the others, but the first two animals were soon discarded, because of disadvantage of using small retinas for study of single cell activities with reference to the ERG. The smaller the retina, the greater the shortcircuiting effect on the ERG of the cut edge of the retina, making the ERG to be recorded smaller. The following is, therefore, the comparison only among the frog, carp and turtle.

The three groups of responses, Group-I, -II and -III,

that were found in the frog were also found in the carp and turtle but with different degrees of ease. In the carp, Group-I, or the responses from ganglion cell axons, and Group-III, or S-potentials, were easily found, but with only occasional chances of obtaining Group-II, or the responses from ganglion cell soma or dendrites. In the turtle, Group-III responses were easily obtained, but the chance of obtaining Group-I and -II was small. Incidentally, in the turtle both luminosity and chromaticity type of responses are found (Fig.4) as in the fish, while in the frog only the luminosity type has been found so far. A new observation on the carp's S-potentials will be mentioned in the next section.

b. Group-III Responses (S-potentials) in the Carp

Three types of S-potentials obtained in the carp are illustrated in Fig.5; the luminosity type in (a) which is found in about 60% of S-potentials, the biphasic chromaticity type in (b) which is found in 25%, and the triphasic chromaticity type in (c) in 15%. It has been shown by MacNichol and Svaetichin (1958) that the effect of adapting light at different wavelengths on the response type (b) is something that is in accord with Hering's opponent color theory. Their observation was confirmed by ours, but the question is raised if the response type (c) could also be accounted for on the basis of the opponent theory. Svaetichin et al. (1961) consider that the triphasic chromaticity type corresponds to the r-g-r type fundamental response curve of a modern Hering-type theory of Judd (1951). This could be tested by use of adapting light: If Svaetichin is correct, the red component should be influenced in size by adapting light of red or blue in a similar way, since the component in the blue region is also a representation of red component, according to Judd. Our experiment appears to show the opposite. As is illustrated in Fig.6, The response to test light of 710mμ is decreased in the presence of adapting light of 700mμ (a), but the same response is augmented not only by yellow adapting light (b) but also by blue (c). The response to a blue test light was also found to behave differently to blue and red adapting light, suggesting that the triphasic chromaticity type response curve is a representation of three independent processes. It is now being carefully examined if there are ganglion cells whose discharge pattern is triphasic with respect to wavelength.

3. COMPONENT ANALYSIS OF THE FISH ERG

The work under this heading was mainly performed by Drs. Murakami and Sasaki, both of whom are listed as assistants in this research project.

a. ERG's in Response to Focal and Non-focal Illumination, and Their Depth Recording

It was demonstrated by Motokawa et al. (1959) that the carp retina, detached from the pigment epithelium and mounted on the indifferent electrode with the receptor side up, gives a characteristic pattern of slow response (Fig.7) to a pipet placed on the distal retinal surface when the retina is scanned with a spot of light across the site of recording. When the light spot is on the recording site, a positive response, which may be called focal slow response, is obtained, but as the spot is moved away from the recording site, the response becomes negative (non-focal slow response). More recently, Motokawa, Yamashita and Ogawa (1961) noticed that this kind of spatial distribution of the slow response in the fish is closely related to the discharge pattern of on/off-type ganglion cells.

It may then be expected that, if recording is made from the vitreal side using the eye cup, the polarity relation of the focal and non-focal slow responses or ERG's should be just the opposite to that found in the isolated inverted retina. However, this proved not to be the case, as earlier pointed out by Motokawa et al. (1959). Fig.8 is an example of depth recording from the carp's eye cup with the vitreous humor drained off. In each record, a focal illumination is followed by non-focal illumination, both of white light. (The focal illumination was a light spot of about 2mm in diameter shone on the recording retinal site. The non-focal illumination was diffuse illumination over the whole retina but the recording retinal site of about 2mm in diameter kept in darkness.) As is seen from the surface record (left top), the ERG is focal positive and non-focal negative. This polarity relation is unchanged throughout depth recording, except an interruption by recording of an S-potential at a depth of 100μ. It was peculiar that the retina turned over also gave nearly an identical depth recording; focal positive and non-focal negative.

A possible explanation of the above observation was that an e.m.f. is developed across the border of the focal and non-focal retinal regions to produce an inflow of current into the focal region across the border, which in turn flows out of the opposite ends of the retina in the focal

region, making these ends positive as compared with the indifferent electrode. However, the explanation proposed in the next section as an alternative appears to be telling more of the truth.

b. Distribution of ERG Components in the Focal and Non-focal Retinal Regions --- Presentation by a Model

In order to make the matter simple, the regular order of presentation will be reversed in the following description, showing a model to illustrate the conclusion first, then followed by some supporting experimental data.

In the model in Fig.9, an ERG layer exists within the retina. The R membrane is a high resistance membrane just back of the distal margin of receptors (Tomita et al., 1960). Because of this high resistance membrane, the polarity of ERG is reversed on the opposite sides of the ERG layer, as shown schematically by rectangular solid lines. The model also shows that the polarity of the focal ERG is opposite to that of the non-focal ERG. Streaming lines carrying arrows in Fig.9A shows that some ERG current originating from non-focal regions flows across the focal region in the direction to cancel the focal ERG proper, and if the focal region is limited to a small area, this could cause polarity reversal of the response in the focal region as shown by dotted lines. The same schema also holds for the model in Fig.9B in which the non-focal region is limited to a small area. It may now be clear that the depth recording in Fig.8 is the result of all of these complications, showing the distribution of potentials shown by dotted lines in Fig.9A and B.

With regard to the focal and non-focal ERG's proper represented by rectangular solid lines, each of them is considered to be the result of the combination of two ERG components, PII and PIII, and is drawn in Fig.9 to show the polarity that is common in the photopic state.

c. Evidence for the Model

(1) Effect of Small Indifferent Electrode

The focal ERG proper in Fig.9A and the non-focal ERG proper in Fig.9B, both of which are shown by solid lines, would be disclosed, if the disturbing effect of current originating in the surrounding could be excluded or reduced. As one of such measures, the indifferent electrode was made small, about 2mm in diameter, and was placed just under the recording site. All the other conditions were the same,

except for the use of the isolated retina. Fig.10A illustrates the result of surface recording from the isolated retina mounted with the vitreal side up. The focal ERG which is the first response is now negative, and the non-focal ERG which is the second is positive. Fig.10B was obtained after the retina was turned over and mounted with the receptor side up on the same small indifferent electrode. As is naturally expected, the polarity is focal positive and non-focal negative.

(2) Effect of Some Chemicals

The view expressed in the preceding section that the ERG, focal or non-focal, is the result of the combination of two components of opposite polarities, PII and PIII, is based on the observation of the effect of two kinds of chemicals; ethyl alcohol as a PIII depressant and sodium azide as a PII depressant. If the view is correct, both focal and non-focal ERG's from the vitreal surface should be made positive by alcohol, while negative by azide. Exactly what is expected is observed as shown in Figs. 11 and 12.

In the meantime, the effect of alcohol was usually reversible, but that of azide was not.

IV. DISCUSSION

A few remarks on Item III-3 "Component analysis of the fish ERG" may be sufficient at this moment when reprints are available of my latest paper entitled "Electrical activity in the vertebrate retina" (Tomita, 1963) in which much is discussed on the other items in this report.

First of all, we do not necessarily consider that the model in Fig.9 is final. Its construction was based on several observations in carefully controlled experiments, but nevertheless, the impression is that the fish ERG is a little too complex to be fully accounted for from observations of a limited number. We rather expect to this model a role as a working hypothesis subserving for attainment of a more complete form.

In the preceding report, evidence was provided that in the frog the S-potential is a phenomenon independent from the ERG. This was based on the observation that the effect of polarizing current across the retina on the ERG was quite independent from that on the S-potential. A similar result was obtained from a similar experiment on the carp, and for this reason the possibility of any contribution of the S-potential to the ERG was neglected in the construction of the model in Fig.9. However, it is felt difficult to assert that the ERG involves no trace of the S-potential, since the S-potential in the fish is so large. In the turtle again, the size of S-potential to be recorded with micropipets could often be predicted from the size of the ERG obtained from the retinal surface. While the problem will be left open to research in the coming year, the characteristic response of the fish S-potential to colored light as shown in Fig.5 might help elucidate the matter concerned.

V. SUMMARY

Attempt at identifying the cell types that make sources of the ERG components is continued from our work described in Final Report on U.S.Army Research Grant No. MG1-60-1. Of the three groups of responses (Group-I, -II and -III) obtained from single units in the frog's retina, only the Group-II responses were left suspected in the preceding report to be constituents of the ERG, but the present work excludes this possibility, since it has become most probable that the responses in question arise from the soma and/or dendrites of ganglion cells which are known to contribute little to the ERG.

The failure to record any constituents of the ERG in the frog has led our research in two directions. The one is the effort to make intracellular recording possible, by some further technical improvement, from cell types other than those already studied but proved not to make ERG constituents. A technique of "harpooning" single cells with pipets in momentary motion at high acceleration is being tested. The other is to apply the conventional technique to materials other than the frog and see whether the localization of ERG constituents is possible in these materials. Among several animals so far examined, the carp is subject to a more thorough test in this report. Studied are properties of Group-III responses (S-potentials) which are related to color reception mechanisms, and the spatial distribution of ERG components which is far more complex than in the frog, due probably to an addition of the color reception mechanisms in the fish. Results are presented by a model.

In the fish the ERG recorded from the region illuminated (focal ERG) is opposite in polarity to that recorded from near-by regions (non-focal ERG). It is demonstrated that both focal and non-focal ERG's involve two major components, PII and PIII, and that in the photopic state the focal ERG is predominant in PIII, while the non-focal ERG in PII. Isolation of the two components is made by use of chemicals.

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VII. ILLUSTRATIONS

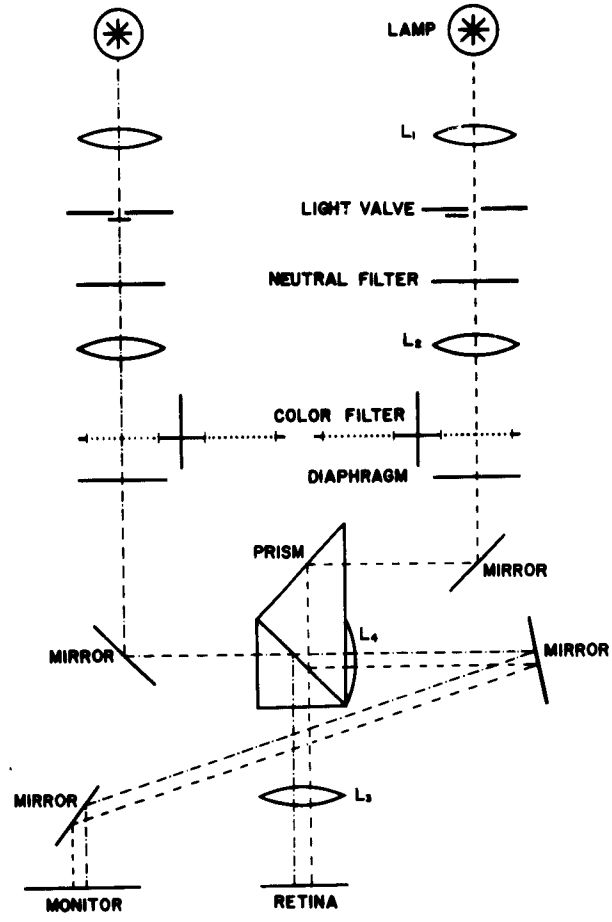

Fig.1. Schematic diagram of two-channel photostimulator. Each channel contains a series of interference filters which cover the visible spectral range in some 20mμ steps and have been adjusted to give colored light of equal energy. (Tomita, Murakami and Hashimoto, 1960).

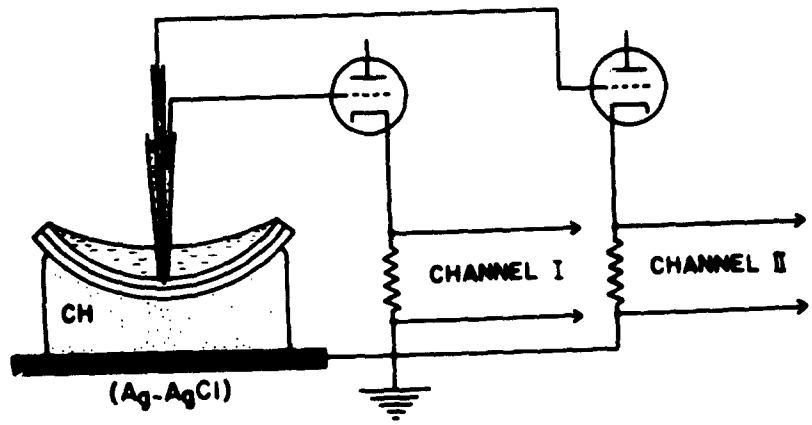

Fig.2. Arrangement for simultaneous recording of surface and intraretinal ERG's with a coaxial microelectrode. Explanation in text.

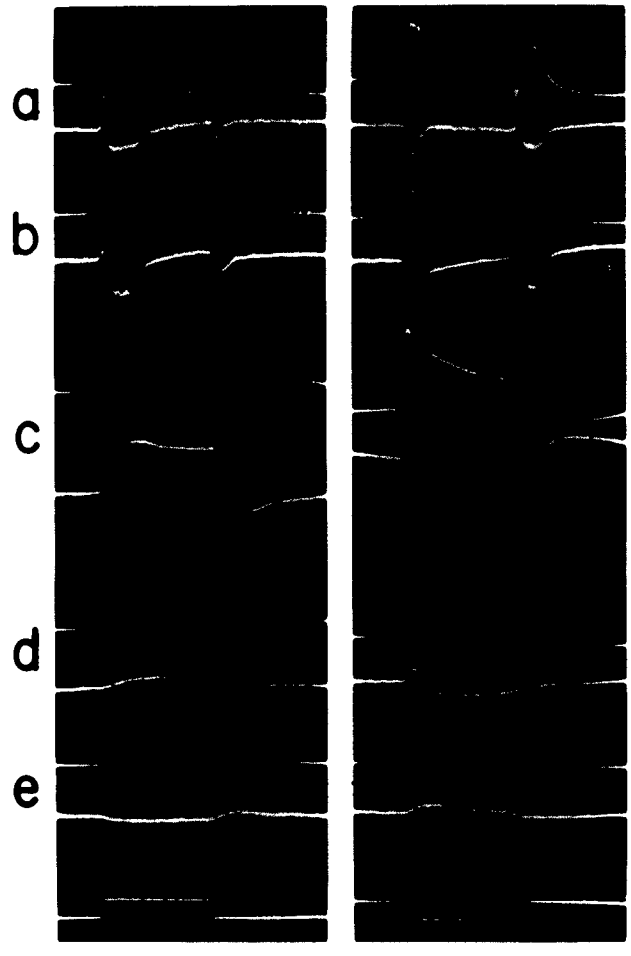

Fig.3. Simultaneous recording of surface ERG (upper tracing of each record) and intraretinal ERG (lower tracing) in the course of azide action. Arrangement in Fig.2 was used. Left column: Responses to on-off of light. Right column: Responses to off-on of light. Further explanation in text.

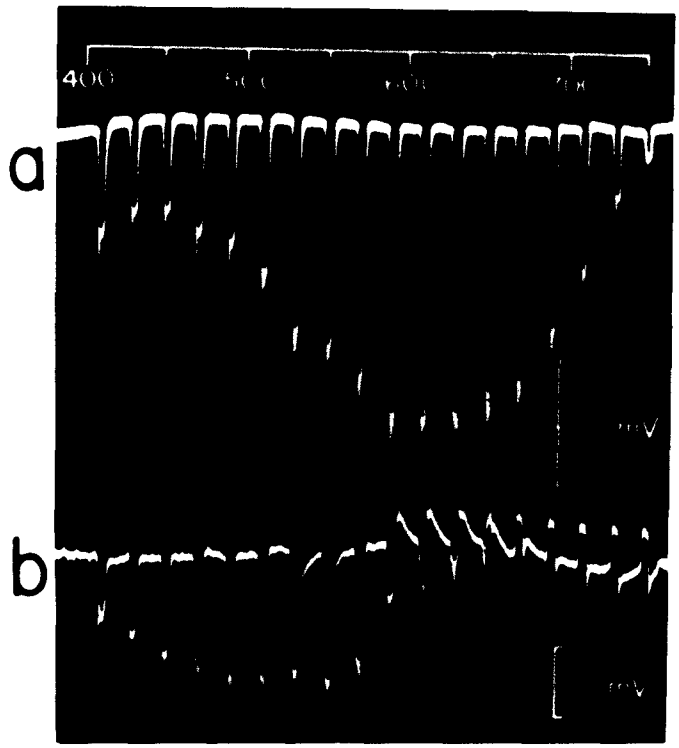

Fig.4. Two types of spectral response curves of the turtle's S-potential; luminosity type (a), and chromaticity type (b). At the top, wavelengths of spectral light are shown in mμ.

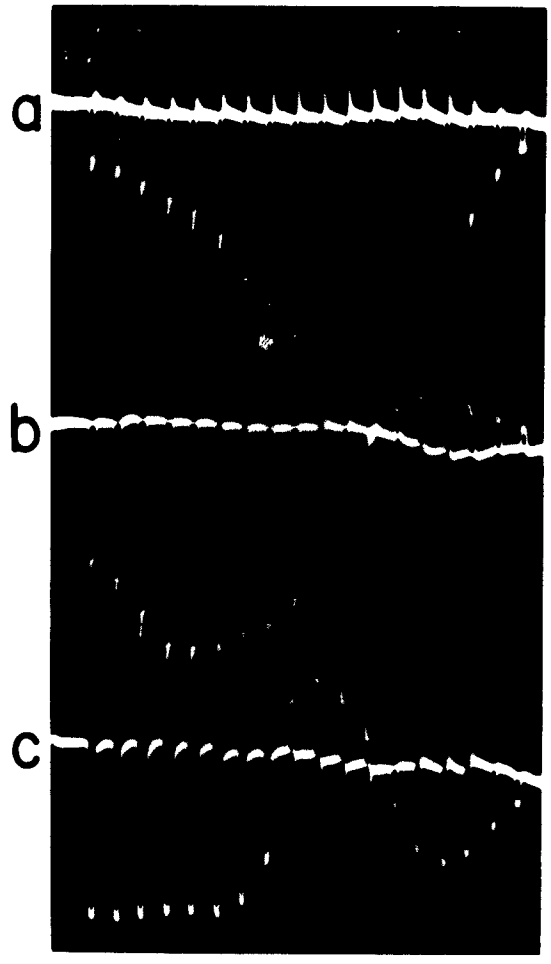

Fig.5. Three types of S-potentials in the carp; luminosity type (a), biphasic chromaticity type (b), and triphasic chromaticity type (c).

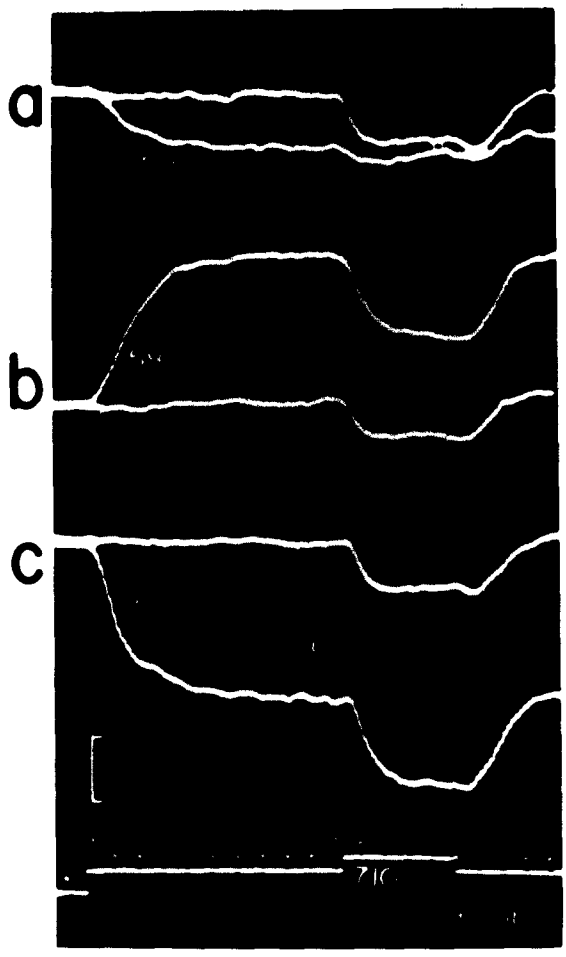

Fig.6. Effect on the response of a triphasic chromaticity type S-potential (carp) to the test light (710 mμ) of adapting light at three different wavelengths; 700 mμ (a), 580 mμ (b), and 400 mμ (c). The onset of adapting light precedes by 1 sec. that of the test light. See text.

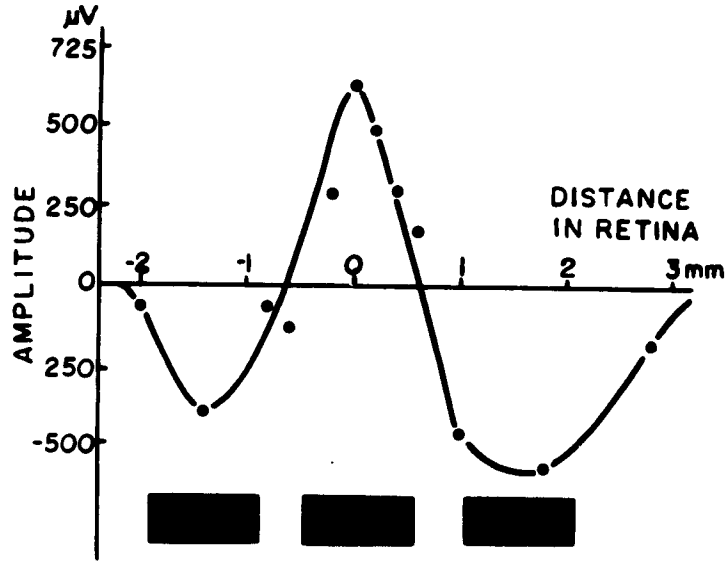

Fig.7. Spatial distribution of the slow response in the carp, obtained from a pipet electrode on the distal retinal surface at point 0 and by scanning with a light spot across the site of recording (courtesy of Motokawa et al., 1959).

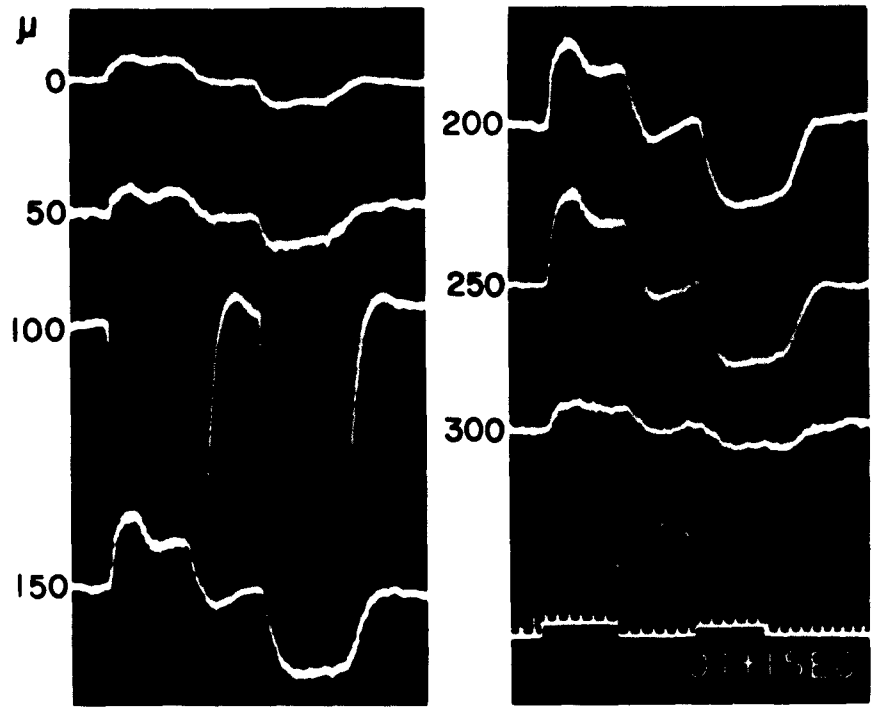

Fig.8. Depth recording from the carp's eye cup with the vitreous humor drained off. Each record is made up of successive two responses; response to focal illumination followed by response to non-focal illumination. Depths of recording from the vitreal retinal surface are shown in micra. Explanation in text.

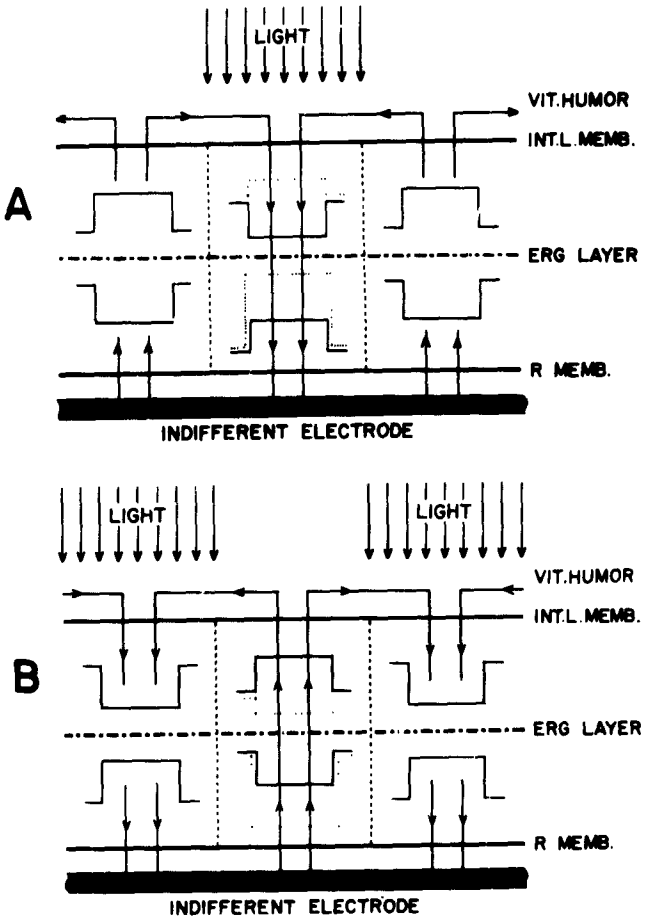

Fig.9. Schematic illustration of the distribution of ERG potential in the fish retina (light-adapted), showing that light produces a response dominant in PIII in the retinal region illuminated (focal ERG), while a PII-dominant response is induced in the nearby retinal region (non-focal ERG). Further explanation in text.

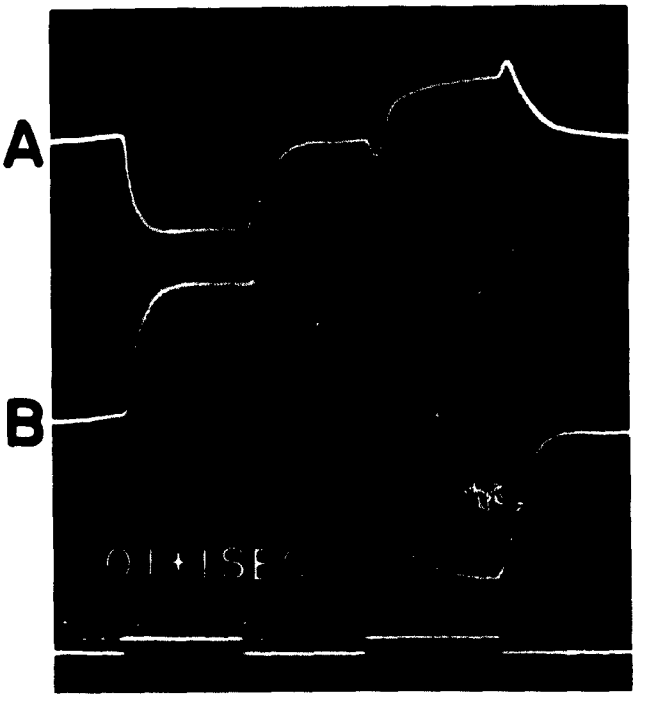

Fig.10. Successive recording of focal response and non-focal response from carp's retina detached from the pigment epithelium and mounted on a small indifferent electrode (2 mm across). Recording micropipet electrode just above the indifferent electrode but across the retina. Record-A was obtained from the retina mounted with the vitreal side up, and B from the retina turned over. See text for further explanation.

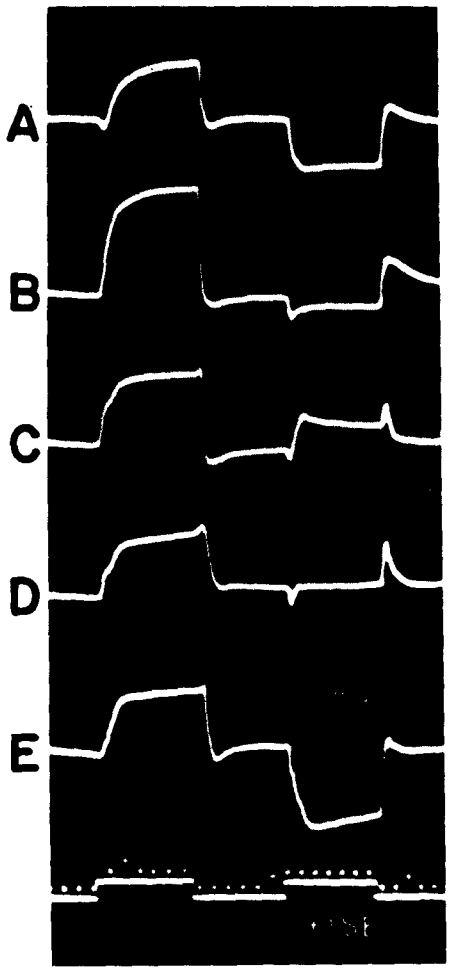

Fig.11. Effect of ethylalcohol on focal and non-focal ERG's successively recorded from carp's eye cup with the vitreous drained off. After recording (A) as a control to show that the ERG is focal positive and non-focal negative, 10% alcohol-Ringer was applied in the eye cup, but drained off immediately. Subsequent records; 3 min. (B), 9 min. (C), 12 min. (D), and 23 min. (E) after alcohol. Both focal and non-focal ERG's are positive in (C), but recovering the initial state in (E).

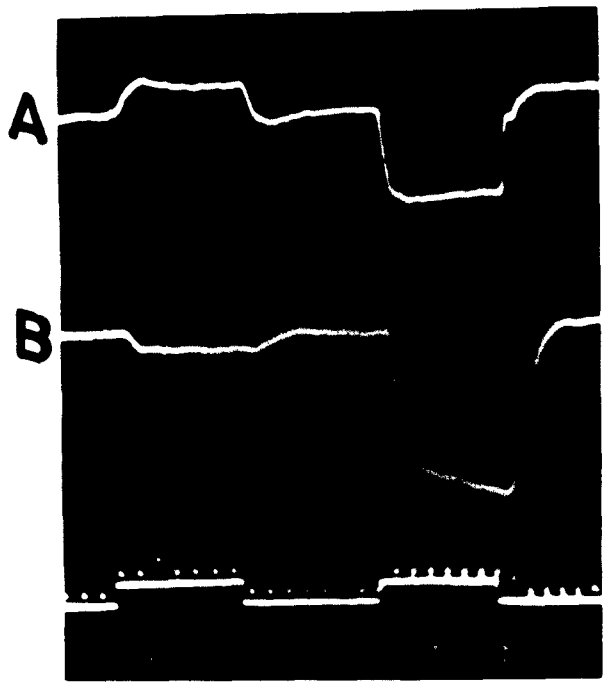

Fig.12. Effect of sodium azide on focal and non-focal ERG's successively recorded from carp's eye cup with the vitreous drained off. After recording (A) as a control, 0.1% azide-Ringer was applied in the eye cup but drained off immediately. Record (B) obtained 4 min. after shows that both focal and non-focal ERG's are negative. The initial state did not recover after azide.